Original Article

Toxicological and Pathological Review of Concurrent Occurrence of Nitrite Toxicity and Swine Fever in Pigs

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ABSTRACT

Background: Plant associated nitrate/nitrite poisoning in buffalo, cattle, goat and sheep had been reported from various parts of the world. Horses and pigs are considered less susceptible to nitrate poisoning. In this study epidemiology of rare outbreak of nitrate poisoning in combination with classical swine fever in a small pig farm was investigated for development of strategies to control and prevent such incidents in future. **Materials and Methods**: Concurrent infection of nitrate toxicity and classical swine fever were recorded in district Nawanshahar, Punjab. Eight pigs suddenly fell sick and died 2 days after feeding barseem + oats and marriage waste food. Twelve pigs were sick exhibiting symptoms of anorexia, fever (104-105°F), depression, constipation followed by diarrhea, respiratory difficulty, tremors and staggering gait with recumbency in four completely off-feed pigs. Blotchy discolorations of the skin of extremities (ears and snout) were observed in three pigs. **Results**: Hematological examination revealed marked leucopenia. Postmortem examination revealed dark brown colored blood evident on opening the carcass and presence of barseem, oats in stomach and intestines. Lymph nodes were swollen and hemorrhagic. Serosal surface of spleen show various infarcts and button ulcers were recorded in cecum and colon, pathognomic lesion of classical swine fever. Nitrate toxicity was confirmed on the basis of quantitative determination of nitrate in the biological material of sick and dead animals. Fodder samples were (barseem + oats) positive for diphenylamine blue (DPB) test, Nitrate concentration in offended barseem and oats were found to be 2612 ppm and 3344 ppm as nitrate nitrogen (No3-N), respectively. Excessive amount of nitrate in stomach contents (924-1365 ppm), liver (22-48 ppm) and kidney (17-22 ppm) of dead animals ($n = 8$) confirmed that death of pigs was due to toxicity induced by nitrate/nitrite. **Conclusion:** The green fodder should be used cautiously in pigs and screening of fodder with DPB test prior offering to animals is strongly recommended to contain the nitrate/nitrite toxicity risk.

Key words: Classical swine fever, nitrate toxicity, swine

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INTRODUCTION

Plants are one of the important causes leading to toxicity in animals in several ways and nitrate/nitrite poisoning is the more likely risk in ruminants but occurs rarely in horses and pigs. Nitrate can accumulate in many crop and pasture plants, weeds and forages viz., berseem (*Trifolium alexandrinum*), bajra (*Pennisetum glaucum*), maize (*Zea mays*), oats (*Avena sativa*), sorghum (*Sorghum vulgare*)

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and toriya (*Brassica napus*).[1,2] In ruminants, when nitrate breakdown system is in balance, nitrate is converted to nitrite by rumen microbes. The nitrite is changed to ammonia that is subsequently used for synthesis of protein and excess ammonia is absorbed by blood and passed in the urine as urea. Ruminants consuming a high nitrate/nitrite fodder/feed, some of the nitrate cannot be immediately converted to nitrite and finally to ammonia resulting into nitrate and nitrite accumulation in the rumen leading to mortalities in ruminants. Plant associated nitrate/nitrite poisoning in buffalo, cattle, goat and sheep had been reported from various parts of the world in literature.[3-9] Horses and pigs are considered less susceptible to nitrate poisoning. These animals convert nitrate to nitrite in large intestine, closer to the end of the digestive tract, where there is less opportunity for the nitrite to be absorbed by blood. But pigs are very susceptible to nitrite poisoning. Nitrite gets absorbed into blood stream and combines with ferrous ion of hemoglobin (Hb), oxidizing it to ferric state resulting into production of met-hemoglobin (met-Hb). Met-Hb has compromised oxygen-carrying capacity, therefore, cannot carry oxygen from lungs to tissues leading to oxygen deficiency in affected animal. Death occurs when level of Met-Hb reaches 60% or higher of total Hb in blood.[10] From literature, we could trace only three case reports of nitrite poisoning in pigs.[11-13]

Classical swine fever is a contagious viral disease caused by a member of the genus Pestivirus of the family flaviviridae and affects pigs of all age-groups. Disease is worldwide in distribution and has been reported frequently from various parts of India causing huge economic losses.[14] Many outbreaks of classical swine fever have been recorded in pigs in India.[15,16] There is no record of plant associated nitrate/nitrite toxicity alone or in combination with swine fever in pigs in India. Therefore, this manuscript reports the occurrence of a rare outbreak of nitrate poisoning in combination with classical swine fever in a small pig farm. The epidemiology of the outbreak was investigated for development of strategies to control and prevent such incidents in future. Factors responsible for occurrence of outbreak were studied and discussed.

MATERIALS AND METHODS

Case history

A pig farm in district Nawanshahar of Punjab was visited on receiving complaint of sudden attack of disease in pigs. The detailed clinical history and epidemiological data of the outbreak was recorded. At a small farm of 35 pigs, animals were kept in open, brick walled sties with roof made up of wood and straw providing shade and shelter. The owner declared that earlier, pigs were being maintained on a dry feed and were eating well and appeared healthy the day before this mishap. On the day of outbreak green feed consisting of barseem and oats was introduced along with left-over food from a marriage party. The green fodder was obtained from field which was fertilized with urea, dung and poultry litter washed from the neighboring poultry farm. The hand-pump was used as source of drinking water for animals. Out of 35 pigs, 30 pigs suddenly fell sick after feeding green feed and left-over food within a period of 12 h. Unaffected 5 pigs were adult boars. Eight pigs died after 2 days of sickness showing brown discoloration of conjunctiva. These eight pigs were subject to complete bacteriological, parasitological, pathological and toxicological investigation in addition to gross examination. Affected pigs were exhibiting symptoms of anorexia, depression, constipation followed by diarrhea, shivering, respiratory difficulty and tremors. All the sick pigs were completely off-feed and four were recumbent. Fever (104-105°F) and staggering gait was recorded in 12 pigs. Three pigs showed blotchy discolorations of the skin of extremities (ears and snout). There was no history of vaccination against swine fever. Local veterinarian treated sick animals with antibiotics and there was no improvement in the health status of animals.

Toxicological studies

Samples of mouldy green fodder (barseem and oats), representing the pigs' last feed and water were collected. The green feed and water sample were tested for nitrate and nitrite levels. For comparison, barseem and oat samples grown in the neighbouring farms of the same village and the University farms were collected and tested for the presence of nitrate and nitrite content. Specimen of food waste mixture, consisting of Naan (Indian bread made up of all purpose fermented wheat flour or maida) and Spinach + Paneer (ripened fermented cheese) curry, was not available for sample collection. Plants were tested for nitrate qualitatively with DPB 1%. Later on nitrate and nitrite were quantified in fodder and water samples by colorimetric methods using spectrophotometer.[17,18]

A provisional diagnosis of nitrate/nitrite mixed with classical swine fever was made on the basis of history, symptoms and PM findings. Blood from 12 severely affected animals and various specimens viz., stomach contents, liver and kidneys of eight dead animals were collected for toxicological and histopathological examination. Blood collected in EDTA tubes was used for hematological analyses and met-Hb levels. Plasma was used to measure nitrite by employing diazotization of sulfanilic acid with nitrite ion and coupling with naphthylethylenediamine to generate pink metabolite and absorbance was measured using spectrophotometer at 540 nm.[18] Met-Hb levels in blood were determined by the method of Smith and Beutler.[19]

Histopathological studies

Tissue samples were collected in 10% buffered formalin. Thin sections of 5 µm thickness were stained with routine haematoxylin and eosin (H and E) stain as per standard protocol of Luna[20] for histopathological studies.

RESULTS

Hematological and toxicological test results

Blood obtained from sick animals was of dark brown color which was pathognomonic of met-hemoglobinemia. Hematological examination revealed marked leucopenia. The met-Hb concentration ranged between 40-66% in blood of affected animals. The mean met-Hb level in unaffected pigs (n = 5) was 15%. The Hb level (4.8-6.6 g/ml) was quite low in the sick pigs compared to concentration of Hb (9.8-10.2 g/ml) in unaffected pigs (n = 5). The affected pigs (12) that showed severe clinical signs of illness exhibited plasma nitrite concentration range of 3.52-7.56 mM. The nitrite levels in plasma of pigs (n = 10) that recovered with treatment were <2 mM and unaffected pigs had <1 mM of nitrite in their plasma. The suspected green feed was tested preliminary by DPB that indicated excessive nitrate in it. Green feed with DPB showed an immediate colour change to dark blackish blue that was suggestive of >2000 ppm nitrates. Nitrate concentration in offended barseem and oats were found to be 2612 ppm and 3344 ppm as nitrate nitrogen (No3-N), respectively [Table 1]. The sample of oats contained high level of nitrite (975 ppm), but barseem showed 435 ppm of nitrite. However, No3-N in barseem and oats collected from the other fields of the same village ranged from 525-850 ppm and all the samples were negative for nitrite. The barseem and oats samples collected from the University fields were also negative for the nitrite. The nitrate and nitrite level in water that was used as drinking water for pigs was 69.6 ppm and 4.5 ppm, respectively.

Necropsy and histological observations

On postmortem examination, there was poorly clotted distinct dark brown color blood in dilated blood vessels of dark brown carcasses. Muscles were also stained brown with blood and there were cardiac hemorrhages and pulmonary congestion in dead animals. Other findings include presence of barseem, oats in rumen and intestines. Hemorrhages in internal organs, spleenomegaly and infarcts on spleen and button ulcers in colon region [Figure 1] of few animals (n = 4) indicated concurrent occurrence of swine fever with nitrate/nitrite poisoning. In these animals lymph nodes were also hemorrhagic and swollen. Histopahological examination revealed ulceration in large intestine characterized by sloughing of intestinal mucosal epithelium [Figure 2] and infiltration of mononuclear cells in the sub mucosa. Liver showed degenerative changes and lymphomononuclear cell infiltration and wedge shaped infarcts and hemorrhages were seen in spleen. In lungs edema, hemorrhages and infiltration of mononuclear cells were reported.

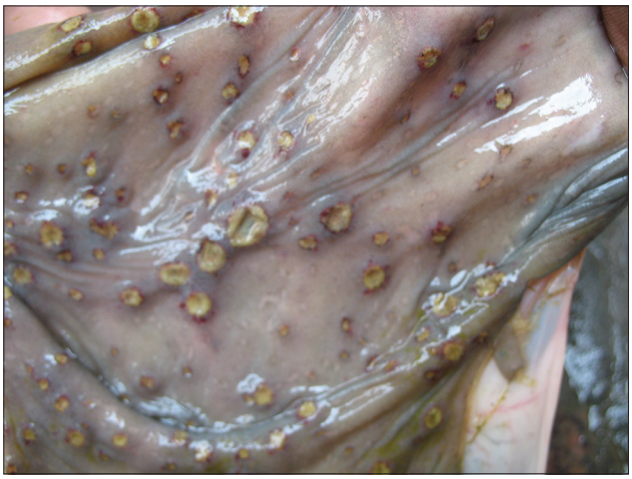

Figure 1: Button ulcers in large intestine

Table 1: Results of toxicological analyses of samples associated with nitrite poisoning in pigs

Sample	Source	Nitrate	Nitrite	Hb (g/ml)	Met-Hb (%)
Barseem (*n*=1)	Pig farm	2612 ppm	435 ppm	-	-
Barseem (*n*=6)	Other felds from same village	525 ppm	Negatve	-	-
Barseem (*n*=5)	University felds	606 ppm	Negatve	-	-
Oat (*n*=1)	Pig farm	3344 ppm	975 ppm	-	-
Oat (*n*=6)	Other felds from same village	850 ppm	Negatve	-	-
Oat (5)	University felds	790 ppm	Negatve	-	-
Water	Pig farm	69.6 ppm	4.5 ppm	-	-
Blood (*n*=5)	Unafected pigs	-	0.55-0.75 mmol	9.8-10.2	12-18
Blood (*n*=12)	Severely afected pigs	-	3.52-7.56 mmol	4.8-5.7	58-66
Blood (*n*=10)	Sick pigs but recovered	-	1.40-1.84 mmol	6.0-6.6	40-48
Stomach contents (*n*=8)	Afected pigs	924-1365 ppm	-	-	-
Liver (*n*=8)	Afected pigs	22-48 ppm	-	-	-
Kidney (*n*=8)	Afected pigs	17-2ppm	-	-	-

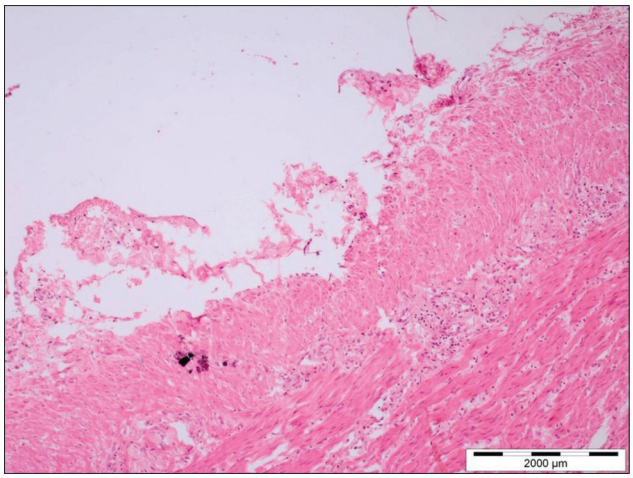

Figure 2: Ulceration in the intestine: Sloughing of the mucosa and cellular infiltration in the sub mucosa

DISCUSSION

Nitrite poisoning in pigs was diagnosed on the basis of clinical signs, laboratory findings (high concentration of nitrite (3.52-7.56 mM) in plasma and met-Hb% =40-66% in affected pigs; excessive amount of nitrate and nitrite in green feed), PM findings and response of affected animals to the treatment. The results of laboratory analyses of green feed that was found to contain huge amount of nitrite confirmed that pigs suffered from nitrite poisoning. Decreased hemoglobin level (4.8-6.6 g/ml) in affected pigs was a consistent finding that also supported the diagnosis. It might be due to the hemorrhages in gastrointestinal tract and other visceral organs and due to the depressive effect of nitrite on bone marrow of pigs.[10,21] Nitrite kills the animal through terminal hypoxia caused by met-haemoglobinaemia. High met-Hb levels reduce the oxygen-carrying capacity of the blood and hypoxia and central nervous system depression precedes death.[10,21] Previous authors suggested that pigs are more susceptible to nitrite toxicity than cattle and sheep because pigs have low level of met-Hb reductase enzyme that is responsible for reversal of methemoglobinemia caused by nitrite poisoning. It has been demonstrated that activity of the enzyme met-Hb reductase varies in different species of animals. The level of this enzyme determines the sensitivity of a species towards methaemoglobin forming substances like nitrites because it is the only system that maintains haemoglobin in its oxygen-carrying reduced state within the erythrocytes.[19,22,23] However, ruminants can tolerate higher levels of nitrates in feed as ruminant microflora convert nitrite into ammonia with the help of nitrite reductase enzyme.[1,21,22] Met-Hb reductase is considered as a rate-limiting enzyme controlling the toxicity of nitrite's effect on the red blood cells. Therefore, ruminants with higher met-Hb reductase activity convert met-Hb back to Hb rapidly than do species (pigs) with lower activity, and will therefore be less susceptible to nitrite induced toxicity. The minimum lethal dose of sodium nitrite is 70-75 mg/kg for pigs than 150-170 mg/kg of nitrites in cow.[21] Fatalities in domestic pigs with nitrite poisoning had been associated with low levels of met-Hb reductase enzyme.[24] It is known that animals can tolerate met-Hb up to 50% without showing any clinical symptoms and death occurs when met-Hb level approaches 80%.[1,21] The pigs in this farm were not accustomed to intake of green fodder containing nitrate/nitrite. This was the reason that pigs died with low levels (40-66%) of met-Hb and Hb (4.8-6.6 g/ml). This agrees with the previous report in which five cows having met-Hb of 41.6% were found dead after consumption of green grass which was irrigated with municipally treated wastewater in Jordan.[9] It supports the previous reports where it has been suggested that met-Hb levels >50% can cause acute toxicity in animals.[8,21] This indicated that previous exposure of livestock to nitrate/nitrite, acclimatizes them to handle it without causing illness. Excessive amount of nitrate in stomach contents (924-1365 ppm), liver (22-48 ppm) and kidney (17-22 ppm) of dead animals (n = 8) confirmed that death of pigs was due to toxicity induced by nitrate/nitrite. We could not trace any reference of tissue concentrations of nitrate/nitrite in pigs poisoned naturally with nitrate/nitrite in literature. However, nitrate tissue concentrations in liver (65.8-172.2ppm) and kidney (39.0-75.0 ppm) had been found in earlier nitrate toxicity outbreaks occurred in bovines.[8]

Further young pigs are more at risk of nitrate/nitrite induced toxicity because they have microflora that can convert nitrates into nitrites.[1] This might be the reason for the sudden death of eight young piglets within 12 h of feed intake and twelve severely affected young ones that died despite treatment. This outbreak which is the first of its kind in India suggested that nitrites can be formed in good amount (435 ppm) in barseem and oats (975 ppm), under unusual circumstances. This suggested that pigs died suddenly due to sudden vasodilation caused by feed rich in nitrites. Similar observations were made in cattle.[9] In this outbreak, fodder was grown with nitrate rich fertilizer, cattle dung and poultry litter slurry. Bacterial and fungal decomposition of the fodder must have added to the concentrations of nitrites as the cut fodder was kept in the sun without shade for 10h during very hot and humid weather. According to Clarke and co-workers[21] normal plant materials may contain high levels of nitrites if attacked by bacteria or fungi or stored in hot and humid conditions. The feeding of marriage waste along with mouldy fodder might have contributed in potentiating the effects of nitrite toxicosis. It can alter the stomach pH to acidic that also favours the conversion of nitrates to nitrites. Button ulcers in large intestine and infarcts in the spleen, pathognomic lesion of CSF[15,25] were also reported in this study. These infarcts are a result of a disrupted blood flow to certain areas resulting from the occlusion of blood capillaries by thrombi.[25] The lower levels of nitrates

and no nitrites in barseem and oat samples of University and other fields of the same village confirmed that faulty agronomical and management factors were responsible for the occurrence of nitrite toxicity in pigs. Nitrate toxicity causes immunosuppression which might have resulted in the precipitation of classical swine fever.

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